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Rhizosphere allocation by canopy-forming species dominates soil CO₂ efflux in a subarctic landscape

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Summary

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- In arctic ecosystems, climate change has increased plant productivity. As arctic carbon (C) stocks predominantly are located belowground, the effects of greater plant productivity on soil C storage will significantly determine the net sink/source potential of these ecosystems, but vegetation controls on soil CO₂ efflux remain poorly resolved.
- In order to identify the role of canopy-forming species in belowground C dynamics, we conducted a girdling experiment with plots distributed across 1 km² of treeline birch (*Betula pubescens*) forest and willow (*Salix lapponum*) patches in northern Sweden and quantified the contribution of canopy vegetation to soil CO₂ fluxes and belowground productivity.
- Girdling birches reduced total soil CO₂ efflux in the peak growing season by 53%, which is double the expected amount, given that trees contribute only half of the total leaf area in the forest. Root and mycorrhizal mycelial production also decreased substantially. At peak season, willow shrubs contributed 38% to soil CO₂ efflux in their patches.
- Our findings indicate that C, recently fixed by trees and tall shrubs, makes a substantial contribution to soil respiration. It is critically important that these processes are taken into consideration in the context of a greening arctic because productivity and ecosystem C sequestration are not synonymous.

Introduction

Climate warming is causing large-scale increases in primary productivity in much of the terrestrial Arctic (Myers-Smith *et al.*, 2020), as predicted by long-term warming experiments (Elmendorf *et al.*, 2012a) and vegetation models (Yu *et al.*, 2017). Where changes in ecosystem productivity are occurring, they are driven by increased growth of tundra vegetation (Elmendorf *et al.*, 2012b; Bjorkman *et al.*, 2018), but also often by an increase in cover and geographical range of deciduous shrub species (Myers-Smith *et al.*, 2011). Aboveground carbon (C) accumulation at northern high latitudes, following increased productivity, is projected to continue into the next century (Qian *et al.*, 2010). There also is clear evidence, from responses of trees to historical changes in climate, and global gradient studies, that arctic and alpine treelines are influenced by climate and that forests will expand if climate continues to warm (Richardson & Friedland, 2009). Poleward and altitudinal shifts of treelines already have been observed in some locations (Wilmking *et al.*,

2006; Harsch *et al.*, 2009; Hofgaard *et al.*, 2013; Hagedorn *et al.*, 2014), although responses are heterogeneous due both to historical and ongoing land use and grazing pressure. Forest expansion in the near future will only influence the tundra close to the present treeline, but significant increases in productivity have also been observed in large parts of the low arctic tundra (Reichle *et al.*, 2018). These subzones are found where deciduous shrub species are present, and often dominant, in the plant community (Walker *et al.*, 2005). Shrubs in the tundra grow taller and expand their spatial range in response to a warmer climate (Myers-Smith *et al.*, 2011, 2019a), and are the most likely plant group to increase in dominance across large areas of the low Arctic in this and the next century (Pearson *et al.*, 2013).

Aboveground biomass in the most productive tundra subzones has increased by $\leq 0.1 \text{ kg C m}^{-2}$ to $c. 0.5 \text{ kg C m}^{-2}$ between 1982 and 2010 (Epstein *et al.*, 2012). However, this stock of biomass C is small compared to soil C stocks. Tundra soils in the majority of the treeless Arctic store $\leq 50 \text{ kg C m}^{-2}$ and the highest densities of C are commonly found in the top 30 cm of the profile

(Kuhry *et al.*, 2013; Siewert, 2018), along with almost all of plant root biomass (Jackson *et al.*, 1996; Iversen *et al.*, 2015). This stock surpasses by far the aboveground C storage even in fully forested boreal (Siewert *et al.*, 2015) and subarctic forests (Hartley *et al.*, 2012). Increasing photosynthetic biomass in the Arctic results in more C entering the ecosystem, and there is much interest in the ecosystem feedbacks that may result (Myers-Smith *et al.*, 2011, 2019b). However, primary productivity is just one facet of the terrestrial C cycle, and the fate of assimilated C also must be understood, on timescales varying from minutes to millennia, to enable a forecasting of future ecosystem C storage.

The task of linking aboveground changes in GPP to total ecosystem storage of C is complex. Most C fixed by arctic vegetation is allocated belowground (Street *et al.*, 2018), where the majority of plant biomass is located (Iversen *et al.*, 2015). GPP can be robustly characterized in tundra based on leaf area and basic meteorological data (Shaver *et al.*, 2007), meaning that GPP may be predicted by changes in aboveground canopy properties that can be detected via remote sensing (Epstein *et al.*, 2012). However, the change in ecosystem respiration with increasing shrub and tree encroachment is much more challenging to predict. For example, tall deciduous shrub species that are structurally similar aboveground (*Betula* and *Alnus*) allocate C belowground very differently in relation to nitrogen acquisition (Street *et al.*, 2018), which may, in turn, affect C turnover rates in the soil. The fate of photosynthesized C within an ecosystem may therefore differ significantly between contrasting arctic plant communities.

Soil CO₂ efflux constitutes the largest component of ecosystem C losses; in many forest systems soil CO₂ efflux comprises, in roughly equal measure, of heterotrophic and autotrophic sources (Bond-Lamberty *et al.*, 2004; Subke *et al.*, 2006). The ratios of heterotrophic to autotrophic contributions to the total soil CO₂ efflux are less well-characterized in tundra (Shaver *et al.*, 2007; Hicks Pries *et al.*, 2015), but this information is required in order to understand C budgets. As tall shrubs and trees represent future plant communities, given further climate change (Pearson *et al.*, 2013), it is particularly important to quantify and understand their contribution to soil CO₂ efflux within their present distribution. Quantifying the contribution of recent plant C inputs to soil CO₂ efflux is technically challenging, usually requiring either destructive methods or isotopic labelling techniques to partition autotrophic and heterotrophic CO₂ sources (Subke *et al.*, 2006). Previous trenching and clipping approaches in these ecosystems have caused considerable disturbance, altered soil thermal and moisture regimes, and have generally only been able to quantify the total contributions of all vegetation, including short-stature species, to ecosystem fluxes (Hartley *et al.*, 2012). Stem girdling halts the delivery of photosynthate from canopies to below ground by disrupting the phloem tissue while limiting the reduction in movement of water to the rest of the plant through the xylem, whereas leaves remain alive. This method therefore makes it possible to identify the contribution of canopy-forming species to soil CO₂ efflux, even where extensive understorey plant communities remain, and provides an unique insight into the role of canopy species and

associated ectomycorrhizal (ECM) fungi in controlling C fluxes from the soil (Högberg *et al.*, 2001).

Alongside plant root respiration, respiration from extraradical ECM mycelium can contribute 15–25% of the total soil CO₂ efflux in boreal and temperate forests (Heinemeyer *et al.*, 2007; Hasselquist *et al.*, 2012; Hagenbo *et al.*, 2019), and ECM mycelial necromass has been linked with fast decomposition (Drigo *et al.*, 2012; Clemmensen *et al.*, 2015). Furthermore, low stocks of soil C in treeline forests (compared to adjacent tundra) may be linked to enzymatic oxidation of organic matter by ECM fungi, as they extract organic forms of N (Bödeker *et al.*, 2014), and to a broader rhizosphere priming effect by birch trees and their symbionts (Hartley *et al.*, 2012). In such a system, where canopy-assimilated C is in high demand for the acquisition of N and other nutrients by symbiotic fungi, a large proportion of soil CO₂ efflux should be linked to the C supply from the canopy. By contrast, tundra willow shrub communities typically grow in riparian zones and in areas of deep snow cover, where soil moisture and mineral nutrient influx is higher than in other tundra types (Nadelhoffer *et al.*, 1991; Sturm *et al.*, 2005), potentially reducing plant investment in ECM fungi (Treseder, 2004). Furthermore, high soil moisture and occasional anoxia are not favourable to many ECM fungi, and can limit their growth within the soil matrix (Lodge, 1989; Wurzbürger *et al.*, 2004; Barnes *et al.*, 2018), thus reducing the demand for assimilated C. Willow shrubs have been widely documented to increase in growth and cover in response to climate change (Tape *et al.*, 2006; Forbes *et al.*, 2010; Myers-Smith *et al.*, 2019a), and therefore it is important to understand C cycling in this ecosystem at present in order to predict changes in the future.

Flux partitioning experiments have seldom been done in arctic ecosystems (Subke *et al.*, 2006), and the relative influence of the canopy has never been elucidated by girdling. The problem of partitioning is exacerbated by the diversity and heterogeneity of tundra and treeline plant communities with contrasting dominant plant species (Walker *et al.*, 2005). Quantitative information on rhizosphere processes in contrasting treeline and tundra plant communities in relation to plant productivity is essential to underpin a better understanding of variations in landscape soil CO₂ efflux. To address these issues, we conducted a girdling experiment at plots across a subarctic landscape in northern Sweden to isolate and test the importance of canopy inputs for soil CO₂ efflux and belowground productivity.

Past experiments that partitioned autotrophic and heterotrophic CO₂ fluxes in boreal and northern temperate forests, using stem girdling and trenching, were in situations where the canopy comprised the majority of leaf area (Högberg *et al.*, 2001; Subke *et al.*, 2006). In a subarctic birch forest, leaf area is likely more equally distributed between canopy and understorey vegetation. In this forest, trenching canopy roots and clipping the understorey reduced soil CO₂ efflux by 50% in peak season (Hartley *et al.*, 2012). We therefore hypothesized (1) that the contribution by canopy dominant trees to autotrophic soil CO₂ effluxes would broadly reflect their contribution to the total leaf area of the community. Furthermore, we hypothesized (2) that autotrophic contribution to soil CO₂ efflux would be lower

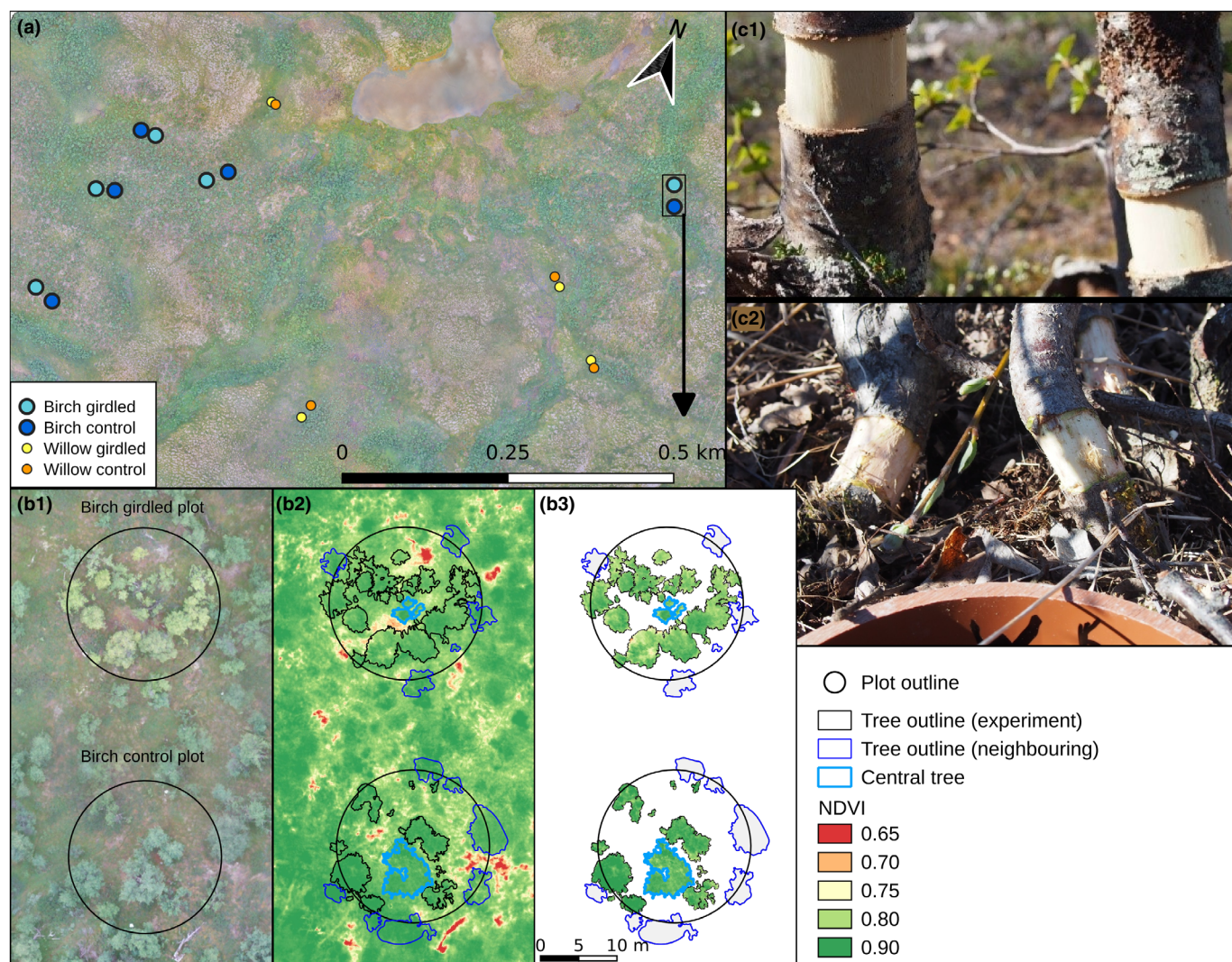


Fig. 1 (a) Location of paired girdled and control plots of birch (blue circles) and willow (orange circles) at field sites south of Abisko (note that one birch pair and one willow pair are 200 m south, out-with the image). (b) Birch pair 6 with plot perimeters superimposed (b1), false colour imagery of Normalized difference vegetation index (NDVI) values and trees within the experiment marked (b2) and experimental and central study tree only marked (b3). (c) Examples of stem girdling in birch plots (c1) and willow plots (c2).

under tundra willow than under treeline forest alongside a lower investment in mycorrhizal fungi.

Materials and Methods

Site selection and experimental design

The experiment was located around a forest–tundra ecotone 3–4 km south of the Abisko Scientific Research Station, Sweden (68°18'N 18°49'E, ~600 m above sea level (asl)). The girdling experiment was carried out in mountain birch forest (*Betula pubescens* Ehrh. ssp. *czerepanovii* (Orlova) Hämet Ahti) and willow thickets (identified as *Salix lapponum* L. but there is very high potential for hybridization in this genus (Forrest, 2006)) that were distributed across a 0.88 km² area (Fig. 1). The birch forests grow on well-drained spodosols, underlain by glacial till without permafrost (Sjögersten & Wookey, 2002). The understorey

primarily comprises ericaceous dwarf shrubs (*Empetrum nigrum* L. ssp. *hermaphroditum* (Hagerup) Böcher, *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L. and *Vaccinium uliginosum* L.) and feather mosses (e.g. *Hylocomium splendens* and *Pleurozium schreberi*) (Supporting Information Fig. S1a). Willow thickets (Fig. S1b) in this area typically grow in poorly drained, late snow-lie communities, alongside *Betula nana* L., with an herbaceous and graminoid understorey.

Before girdling, five willow and six mountain birch plots were established in early June 2017, with each plot divided into paired subplots. Pairs were selected to have similar tree and stem density, soil carbon (C) stocks, soil C : nitrogen (N) ratio (Table 1) and understorey (birch plots: ericaceous dwarf shrubs and mosses; willow plots: forbs and mosses). The birch subplots had a circular area with a radius of 10 m and an average tree density of 586 trees ha⁻¹ (Table 1). The willow subplots had a radius of 2 m and a density of 5–6 stems m⁻², representing the largest

Table 1 Average (± 1 SE) vegetation and soil characteristics in control and girdled plots of birch ($n = 6$ pairs) and willow ($n = 5$ pairs).

	Birch		Willow	
	Control	Girdled	Control	Girdled
Trees (trees ha ⁻¹)	573 \pm 72.1	600 \pm 83.6		
Stems (stems m ⁻²)	0.27 \pm 0.03	0.20 \pm 0.01	5.98 \pm 0.45	5.52 \pm 0.43
Canopy height (cm)			82.7 \pm 9.26	76.7 \pm 8.57
Organic horizon SOC* (kg m ⁻²)	2.80 \pm 0.17	2.57 \pm 0.20	3.07 \pm 0.46	2.50 \pm 0.35
Soil C : N ratio	29.6 \pm 1.02	28.7 \pm 1.33	25.0 \pm 0.38	25.6 \pm 1.09
Soil CO ₂ efflux (μ mol m ⁻² s ⁻¹)	2.80 \pm 0.15	2.59 \pm 0.18	2.48 \pm 0.34	2.37 \pm 0.10

Soil respiration values are for measurement days before implementation of the girdling treatment.

*SOC, soil organic carbon.

plots with willow-only canopies that could be found in the study area. The larger size of the birch plots was necessary to ensure that all trees were girdled that could potentially be contributing to belowground respiration at the central measurement area. The outer perimeters of paired birch subplots were separated by 10–20 m and the paired plots were separated from other pairs by between 300 to 1100 m (Fig. 1a). For the willow plots, the distances between paired subplot outer perimeters were 2–16 m and pairs were separated by between 300 and 900 m. Each birch plot contained three to four trees within the central 3 m radius, within which all subsequent measurements were taken. Unlike in the relatively sparse birch forest, the lack of gaps between willow shrubs meant that it also was necessary to trench the perimeter of each willow plot, and plastic sheet was then inserted through the entire soil depth until rocks were encountered (top 10–30 cm of soil) to prevent roots from adjacent plants from entering. Trenching was not carried out around the birch plots, because the size of the buffering area around the central 3 m radius was deemed sufficient to minimize edge effects.

Soil CO₂ efflux measurements (see Soil CO₂ Efflux section for methods) were carried out twice at all birch and willow plots after snow-melt (9–12 June 2017) but before the application of girdling treatments. Paired Student's *t*-tests were carried out to test for a pre-girdling difference in plot characteristics between subplots and no significant differences were observed (Table 1). One subplot of each pair was girdled between 12 June 2017 and 15 June 2017. All birch stems > 1 cm in diameter were girdled within the 10 m radius plot. In the birch plots, a 4–8 cm section of the bark was removed around the circumference of each stem down to the xylem *c.* 30 cm from the ground, leaving no phloem connection between leaves and roots (Högberg *et al.*, 2001). In the willow plots, every willow stem was girdled *c.* 10–20 cm above the ground. Re-sprouting shoots from below the girdle-line were removed by hand whenever observed during the experiment. Birch and willow plants retained leaves until natural senescence in 2017 and all birch trees produced full leaves above the girdle-line in spring 2018. However, girdled willow canopies failed to produce leaves in 2018.

Soil CO₂ efflux

Two days before the first efflux measurement and 5–7 d before girdling, three 5 cm tall, 15 cm inner-diameter, PVC collars were secured within 1 m of one of the three central trees of each birch plot. Collars were placed between understorey stems and areas of moss mats in order to exclude live aboveground plant material. The collars were pushed firmly onto the soil and secured to the ground with nonsetting plumber's putty (Evo-Stik Plumber's Mait[®]), to provide a good seal between collar and soil surface without severing shallow roots. The same method was applied to willow plots, with three collars placed within the central 1 m radius of the plot. Effectiveness of the collar seal using plumber's putty has been demonstrated by a linear increase in CO₂ concentrations when a closed chamber is attached to the collar (Parker *et al.*, 2015).

After girdling, CO₂ efflux was measured on 10 dates in the birch plots and nine dates in the willow plots during the 2017 growing season, and 10 times each during the 2018 growing season. An EGM-5 infrared gas analyser (PP Systems International, Amesbury, MA, USA), with an attached CPY-5 darkened chamber, was used to measure soil CO₂ efflux (root-associated and heterotrophic activity). CO₂ efflux was calculated based on the linear increase in CO₂ concentration over 90 s. For each measurement date, all plots were visited on the same day between 09:00 h and 18:00 h. The order in which plots (species and girdling treatment) and collars were sampled was alternated at every sampling day in order to minimize temporal sampling bias. The average soil CO₂ efflux value from the three collars was recorded as the true replicate flux per plot.

Soil and vegetation characteristics

In order to understand the effects of girdling on soil CO₂ efflux we measured plant and soil characteristics in the different plots. Soil organic C stocks of the organic horizon at each plot were calculated from a mean of nine soil cores (3.8 cm diameter) taken evenly across a 2 \times 2 m area in the centre of the plot. The organic horizons from each core were separated from the lower mineral horizons, mixed together, oven-dried (60°C) and weighed. Carbon and N contents were measured on the combined sample in a Flash Smart[™] elemental analyser (ThermoFisher Scientific, Waltham, MA, USA). Canopy leaf area index (LAI) was measured using an Accupar LP-80 leaf area meter (Pullman, WA, USA) in early August in 2017 (all plots) and 2018 (birch plots only). At the birch plots, an average of 20 measurements taken evenly at 30 cm height across the N–S diameter of the plot was used. In the willow plots LAI was measured at 30 cm height at five points across the plot. The understorey LAI of each birch forest plot was estimated from the average normalized difference vegetation index (NDVI) of the visible forest floor in the 10 m radius of the plot from a drone platform according to relationships from a previous remote sensing study at 3 m scale at a nearby forest-tundra ecotone site ($LAI = 0.00059 e^{9.502 NDVI}$ ($R^2 = 0.90$) (Williams *et al.*, 2008)).

Drone imagery was taken on the 2 August 2017 and 30 July 2018 in two flights using a senseFly eBee mapping drone (SenseFly Inc., Cheseaux-sur-Lausanne, Switzerland) carrying a Parrot Sequoia multispectral sensor that delivers imagery in four spectral bands (Green, Red, Red Edge, Near Infrared) and a separate RGB orthophoto. The drone was operated at a target elevation of 106 m resulting in an effective ground resolution of 10.3 cm (2017) and 11.2 cm (2018) in the final processed raster data of each flight. We used the PIX4DMAPPER photogrammetric software (v.4.2.15; Pix4D, Lausanne, Switzerland) to combine individual images into continuous raster maps. We extracted an orthophoto and the NDVI, which is considered an indicator of vegetation abundance and health (Rouse *et al.*, 1974). Each plot was marked visually in the field and later identified and outlined in the orthophoto composite using 4 m and 20 m diameter circles for willow and birch plots, respectively. The orthophoto was used to digitize manually the outline of the canopy of each individual tree in the birch plots and of the willow shrub coverage in willow plots. For birch plots, we extracted NDVI pixel values for the most centrally located trees in each plot. Understorey NDVI pixel values per plot were extracted from within each circle after masking out all tree canopies.

With every soil CO₂ efflux measurement from 24 July 2017 onwards, conductivity of the top 5 cm of soil was measured at all plots using a handheld HH2 ThetaProbe soil moisture meter (Delta-T Devices, Cambridge, UK). In birch plots, measurements were taken every meter in a 9 m² central square grid (16 measurements), and in the willow plots nine measurements were taken in a 4 m² square grid. Soil temperature at 5 cm depth was measured three times across the grid using a hand-held digital thermometer. Average temperature and conductivity values were calculated for each plot on each sampling date, then conductivity was converted to gravimetric moisture content according to:

$$\text{Gravimetric moisture (\%)} = e^{(a+Mb)}$$

where M is the soil conductivity measured in the field, and a and b are estimated based on the fitted relationship between gravimetric moisture and soil conductivity measured during a dry-down curve of saturated ericaceous peat from near the study plots ($a = 4.402$, $b = 0.00129$, adjusted $R^2 = 0.955$). Using the bulk density of the calibration soil, gravimetric moisture was converted to volumetric moisture. To capture continuous volumetric soil moisture and soil temperature dynamics through timespan of the experiment, EC 5 soil moisture and TMB temperature smart sensors (Onset, Bourne, MA, USA) were installed at 5 cm depth in one birch and one willow plot. The probes logged hourly measurements to HOBO microstation loggers (Onset).

Growing season root production and birch copy numbers

Root production over the growing season was estimated using ingrowth bags (Sullivan *et al.*, 2007). Cylindroid fibre-glass mesh bags (6 cm deep and 2.5 × 1.5 cm wide with a mesh size of 2 mm) were loosely packed with ericaceous peat. The peat was collected within the study landscape, dried for 48 h at 85°C, and

sieved through a 4-mm mesh, with remaining roots picked out by hand before deployment in the bags. Ensuring maximum contact with the native soil, a root ingrowth bag was inserted vertically into the top six cm of organic soil, below the litter and moss layers, 30 cm from every CO₂ efflux collar in the willow and birch plots. Bags remained in the soil from 14 June 2017 until 18 September 2017, and new bags were inserted from 2 June 2018 until 12 September 2018 with a total of 96 and 102 d field incubation per respective growing season. Bags were retrieved from the soil by carefully running a scalpel around each bag to a depth of 6 cm. Outside portions of in-grown roots were cut off in the lab and all roots inside the core were extracted, washed and dried at 60°C for 72 h, after which dry mass was recorded. The C content of the roots was then analyzed using a Flash Smart™ elemental analyser.

For a species-specific assay, subsamples (0.7–30 mg (depending on amounts remaining after other analyses)) of dried ingrowth roots from birch plots were finely milled by steel nuts (40 s at 5000 rpm) in 2-ml tubes (Precellys, Bertin Instruments, Germany), and DNA was extracted using the NucleoSpin soil kit (Macherey-Nagel, Düren, Germany). Copy numbers of the ITS region of *Betula* sp. were analyzed by quantitative PCR (qPCR) using birch-specific primers (ITSb_F and ITSb_R) and a Biorad iQ5 real-time PCR detector system (Bio-Rad) according to Pérez-Izquierdo *et al.* (2019). Two 2017 root samples from girdled plots were not extracted due to lack of sample material at this stage. Tests with known amounts of plasmid DNA and corresponding M13 primers (Pérez-Izquierdo *et al.*, 2019), using the same PCR conditions, indicated no significant PCR inhibition by the root extracts.

Hyphal production

Ectomycorrhizal (ECM) fungal hyphal production over the growing season (same dates as root bags) was estimated using sand-filled ingrowth bags (Wallander *et al.*, 2013). 5 × 5 cm nylon mesh bags with a 37 µm mesh size were filled with 18 g sand from Lake Torneträsk (Parker *et al.*, 2015). The sand was sieved to select particle sizes between 0.125 and 1 mm and autoclaved twice, then dried at 100°C for 72 h. Bags were designed to be thinner than common practice (only 0.5-cm thick when filled) to limit the distance that mycorrhizal fungi had to grow in order to colonize the sand, and to encourage fungal groups that may not typically grow into sand to colonize (Hagenbo *et al.*, 2018). Bags were inserted into the ground at a 45° angle, directly below the litter layer, 30 cm from each CO₂ efflux collar but on the opposite side to the root ingrowth cores. Before insertion, bags were wetted with deionized water on a solid surface in order to ensure uniform sand depth across the bag. Bags remained in the field over the same period as the root bags and blanks were maintained in the laboratory. Sand was extracted from bags 4–6 h after recovery from the field, frozen at –80°C, and freeze-dried for 72 h in a ModulyoD freeze drier (ThermoFisher Scientific). Then 1.5 g sand from each bag were sonicated in 25 ml of deionized water for 10 min in order to free hyphae from the sand. A 10-ml aliquot of the hyphae-containing solution was transferred to a

Falcon tube, to allow further separation of hyphae and sand by sedimentation, then transferred into an open container, dried at 50°C, weighed and analysed for C content using a Flash Smart™ elemental analyser. This process was repeated for eight blank samples that had not been deployed in the field and the average C content was subtracted from all samples.

Statistical analysis

The effects of girdling, species (willow or birch) and season (early, mid and late) on soil CO₂ efflux, soil moisture and temperature were analyzed using linear mixed effects models with the NLME package in R (Pinheiro *et al.*, 2016; R Development Core Team, 2016). In the linear mixed effects model, 'plot' was designated as a random variable, to account for the paired design of the experiment, as was 'sub-plot', to take account of repeated measures. Soil CO₂ efflux immediately after girdling treatment in June 2017 was not considered in the analysis, as it was assumed that the treatment had not yet taken effect. All flux data were natural-log transformed in order to conform to the assumptions of the parametric analysis. The effect of girdling on root and hyphal production, LAI, canopy NDVI and understorey NDVI in birch and willow plots also was analyzed using linear mixed effects models after natural-log transformation when appropriate (except NDVI, which required arcsine-square root transformation in order to be appropriate for parametric analysis).

Data statement

Underlying data will be freely accessible in due course but in the meantime, please contact the corresponding author for access to data.

Results

Across all plots, birch had significantly higher soil CO₂ efflux rates than willow plots in 2017 ($P=0.005$; Fig. 2) but not in 2018. Girdling significantly reduced soil CO₂ efflux in both 2017 ($P<0.001$; Fig. 2) and 2018 ($P<0.001$; Fig. 2) in birch and willow plots compared to paired control plots. This reduction in soil CO₂ efflux was large and sustained throughout the peak seasons of 2017 and 2018. The effect of girdling was maintained into late season (September), although not as pronounced then as in mid-season. The girdling treatment did not have a detectably larger effect on soil CO₂ efflux in the birch plots compared to the willow plots in either year, with a statistically non-significant interaction term between species and treatment ($P=0.38$ and $P=0.11$ in 2017 and 2018, respectively; Fig. 2).

The girdling treatment allowed for the estimation of 'canopy-linked' soil CO₂ efflux (the difference between control and girdled plots) as a proportion of the total soil CO₂ efflux in the control plots (Figs 2, S2). The remaining proportion of the total flux constituted respiration of free-living heterotrophs and remaining roots (understorey and canopy species roots that were still alive). Over the 2017 growing season, the average contribution from

canopy-linked sources to total soil CO₂ efflux in the birch plots was 33%, but this increased markedly to 53% during the peak growing season in early August (Figs 2, S2). In 2018 the average canopy-linked contribution to soil CO₂ efflux was again 33%, with a maximum in early August of 46%. The canopy-linked contribution to soil CO₂ efflux in willow shrub plots was smaller, but still considerable, with an average of 26% (in 2017) and 21% (2018), and maximum contributions of 38% and 30%, peaking in early August in each of the respective years.

Girdling significantly reduced total root production compared to control plots in 2017, for birch and willow combined (willow: −30% change, birch: −75% change; $P=0.009$) (Table 2; Fig. 3), with no significant difference between species ($P=0.834$). This difference was lost in 2018, with no significant effect of species or girdling treatment on root production. However, girdling caused a highly significant reduction in birch ITS copy numbers in ingrowth bags in 2018 ($P=0.004$) (Table 2; Fig. 4), with birch root production decreased to almost zero in girdled plots. Girdling also tended to reduce birch copy numbers during the first growing season of the treatment (2017) ($P=0.079$) (Table 2; Fig. 4).

Although birch control plots tended to have higher hyphal production than girdled plots or willow plots in 2017, there was no overall effect of girdling and only a marginally significant difference between birch and willow plots ($P=0.059$) (Table 2, Fig. 5). By 2018, however, there was a highly significant effect of girdling on hyphal production owing 99% reduction in girdled birch plots ($P<0.001$) (Table 2; Fig. 5). The lack of difference between girdled and control in 2018 in willow plots was associated with a significant interaction between treatment and species ($P<0.001$).

Willow plots had significantly lower NDVI than birch canopy, despite no significant difference in LAI (Table 2), likely due to the pubescent leaves of *S. lapponum*, which reduce reflectivity (Street *et al.*, 2007). Girdling significantly reduced canopy NDVI of both species, but more so in the willow plots, resulting in a significant interaction between species and treatment (Table 2). In 2017, despite differences in canopy NDVI, LAI remained unaffected by girdling with no differences between species. In 2018, LAI in girdled birch plots ($0.65 \text{ m}^2 \text{ m}^{-2}$) dropped significantly below control values ($0.92 \text{ m}^2 \text{ m}^{-2}$) due to reduced birch leaf development in girdled plots ($P=0.024$; Table 2). Understorey NDVI under birch was the same between girdled and control plots in both 2017 and 2018, on average remaining at 0.77. The average LAI of the understorey of $0.88 \text{ m}^2 \text{ m}^{-2}$, estimated from NDVI according to the relationship between ground vegetation LAI and NDVI at a 3-m scale at a nearby site ($\text{LAI} = 0.00059 e^{9.502 \text{ NDVI}}$; Williams *et al.*, 2008), indicated that birch trees contribute approximately half of the leaf area in this ecosystem. It was not possible to make this calculation in the willow plots due to the resolution of the imagery, making it hard to differentiate willow and understorey from the drone platform.

Soil moisture varied significantly between vegetation types ($P<0.001$; Fig. S3b). In the growing season of 2017, soil moisture was 1.6 times higher in willow plots than in birch plots and in 2018 it was 1.5 times higher. There was no statistically

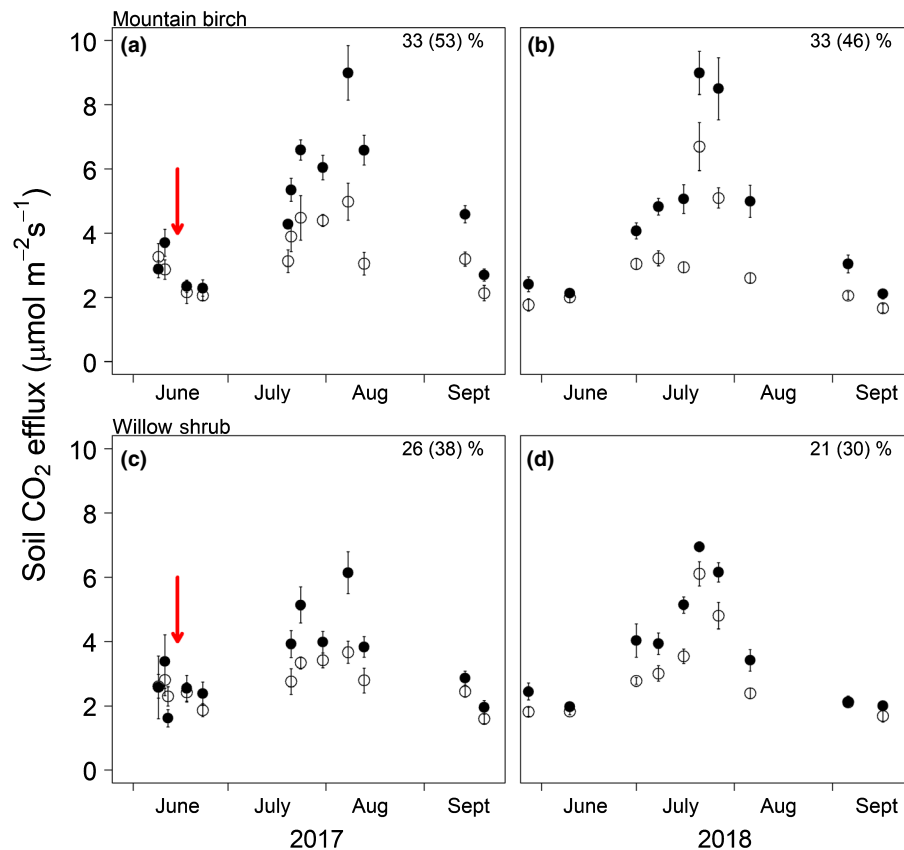


Fig. 2 Soil CO₂ efflux from mountain birch (a) 2017 and (b) 2018, and willow shrub in (c) 2017 and (d) 2018, in control (closed circles) and girdled plots (open circles). Points represent mean values at each sampling date (± 1 SE). Arrows (red) indicate the date of girdling in the respective communities. In 2017 there were significant effects of species ($F_{(1,9)} = 14.0$, $P = 0.005$), girdling treatment ($F_{(1,10)} = 24.3$, $P < 0.001$) and season ($F_{(1,186)} = 130$, $P < 0.001$) and no interactions between species and treatment ($F_{(1,9)} = 0.59$, $P = 0.46$). In 2018 there was no significant effect of species ($F_{(1,9)} = 3.24$, $P = 0.11$) but effects of girdling treatment ($F_{(1,10)} = 36.7$, $P < 0.001$) and season ($F_{(2,196)} = 168$, $P < 0.001$); there was no interaction between species and treatment ($F_{(1,9)} = 2.00$, $P = 0.19$). The percentage contributions of the canopy to soil CO₂ efflux over the over the whole growing season and at its seasonal maximum (in brackets) are reported in the top right of each panel.

detectable effect of girdling on soil moisture in either year. Both willow and birch plots were exposed to a flush of water at the time of snow melt in May/June, but soon after soil moisture dropped to distinctly lower levels in birch plots until soil freeze-up in November (Fig. S3b). Soil temperature was not different between species or girdling treatment (Fig. S3a).

Discussion

Mountain birch forests and willow shrub patches are amongst the most productive ecosystems in the Fennoscandian subarctic and are representative of plant communities that are expanding onto tundra as northern latitudes warm (Myers-Smith *et al.*, 2011; Hofgaard *et al.*, 2013). Although expansion of forest and shrub communities is expected to increase gross primary productivity there is little understanding of how vegetation change will influence the carbon (C) dynamics of the whole system, primarily because the subsequent fate of assimilated C is so poorly quantified and understood (Street *et al.*, 2018). Here, we use a girdling experiment to show that recently fixed C contributes 53% and 33% (peak season and full season, respectively) to soil CO₂ efflux in mountain birch communities, and 38% and 26% to soil CO₂

efflux in willow communities. The results suggested that much of the C fixed into these relatively productive ecosystems is rapidly returned to the atmosphere, constituting a significant fraction of soil CO₂ efflux.

We found that leaf area of the birch canopy (measured here at 0.5–0.92 m² m⁻² depending on sampling year) was approximately the same as the leaf area of the understorey (~0.88 m² m⁻² based on conversion from normalized difference vegetation index (NDVI)). The understorey of subarctic (Kulmala *et al.*, 2019) and boreal (Wardle *et al.*, 2012) forests can contribute 50% of gross primary productivity (GPP), and exclusion of all autotrophic C inputs to the soil in a subarctic birch forest (both canopy and understorey) resulted in a ~50% reduction in soil CO₂ efflux at peak growing season (Hartley *et al.*, 2012). We therefore hypothesized that the contribution from canopy assimilation to autotrophic soil CO₂ fluxes in mountain birch would reflect its contribution to community leaf area, which would equate to an approximate 25% reduction following girdling given the broadly equal leaf area index (LAI) of overstorey and understorey vegetation. Thus, our finding of a 33% reduction in soil CO₂ efflux during the growing season following cessation of inputs from only the birch canopy disagrees with our

Table 2 Mean (± 1 SE) root and hyphae production over full growing seasons, canopy leaf area index (LAI) and understorey Normalized Difference Vegetation Index (NDVI) in late July in birch and willow, girdled and control plots (birch, six paired plots; willow, five paired plots) in 2017 and 2018.

	Birch forest		Willow shrub		Species			Treatment			Species \times Treatment		
	Control	Girdled	Control	Girdled	df	F	P	df	F	P	df	F	P
2017													
Roots (mg C per bag)	11.5 \pm 3.79	2.75 \pm 0.83	6.30 \pm 1.15	4.37 \pm 1.27	1,9	0.05	0.829	1,9	11.1	0.009	1,9	2.50	0.148
Birch ITS* copies (per bag)	6032 \pm 3678	180 \pm 116						1,3	6.87	0.079			
Hyphae (mg C per bag)	1.07 \pm 0.39	0.19 \pm 0.07	0.11 \pm 0.03	0.24 \pm 0.13	1,9	4.64	0.060	1,9	1.35	0.275	1,9	0.95	0.356
Canopy LAI (m ² m ⁻²)	0.50 \pm 0.21	0.58 \pm 0.23	1.51 \pm 0.50	0.56 \pm 0.15	1,9	0.41	0.537	1,9	3.87	0.081	1,9	3.93	0.079
Canopy NDVI	0.87 \pm 0.01	0.85 \pm 0.01	0.83 \pm 0.01	0.77 \pm 0.01	1,9	47.41	<0.001	1,9	122	<0.001	1,9	32.94	<0.001
Understorey NDVI	0.81 \pm 0.02	0.81 \pm 0.01						1,5	0.14	0.724			
2018													
Roots (mg C per bag)	8.84 \pm 2.19	7.15 \pm 1.55	17.41 \pm 5.79	7.68 \pm 2.48	1,9	1.48	0.255	1,9	2.90	0.123	1,9	0.84	0.383
Birch ITS* copies (per bag)	30000 \pm 25317	18 \pm 17						1,5	24.72	0.004			
Hyphae (mg C per bag)	2.43 \pm 0.87	0.02 \pm 0.01	0.32 \pm 0.18	0.18 \pm 0.06	1,9	1.30	0.284	1,9	28.86	<0.001	1,9	21.58	0.001
LAI (m ² m ⁻²)	0.92 \pm 0.10	0.65 \pm 0.07						1,5	10.33	0.024			
Canopy NDVI	0.81 \pm 0.01	0.74 \pm 0.01	0.72 \pm 0.01	0.65 \pm 0.02	1,9	39.92	<0.001	1,9	68.33	<0.001	1,9	0.29	0.606
Understorey NDVI	0.74 \pm 0.02	0.74 \pm 0.02						1,5	0.00	0.964			

Root production values are from the top 6 cm of soil, hyphae production from the top 3.5 cm of soil. Test statistics from linear mixed effects models for fixed effects (species, treatment, and the interaction, if present) for each response variable in each year are provided in line.

*ITS, internal transcribed spacer.

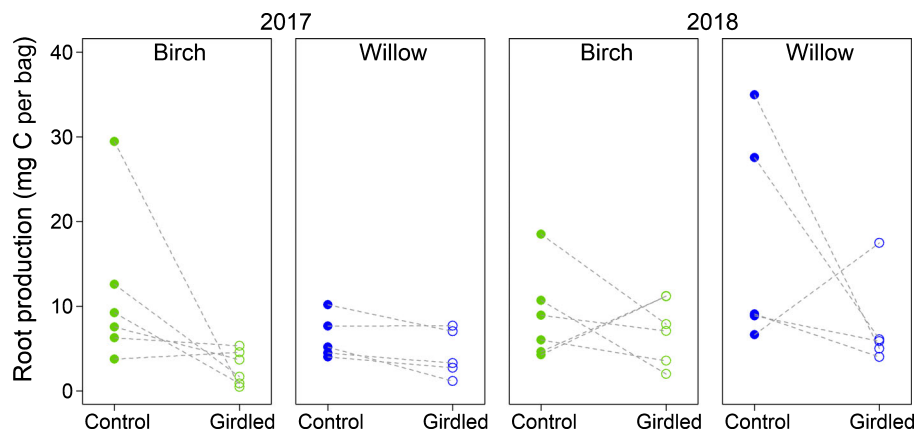


Fig. 3 Root production (mass of carbon (C) into in-growth bag) in paired (denoted by dashed connecting lines) control and girdled plots in 2017 and 2018 in birch (green) and willow (blue) plots. The results of statistical analyses are shown in Table 2.

hypothesis and suggests that birch makes a larger than expected contribution to soil CO₂ fluxes. In the wider context of autotrophic-heterotrophic soil CO₂ efflux partitioning (broadly 50% autotrophic; Subke *et al.*, 2006), the relative contribution of one species which is only one half of the ecosystem leaf area also is remarkable.

The peak season 53% reduction in soil CO₂ efflux with girdling in early August roughly coincides with peak vegetation productivity (Heliasz *et al.*, 2011). Although phenology of peak belowground allocation will vary from year to year, we suggest that belowground allocation scales with increasing aboveground assimilation. The scale and seasonality of the canopy-driven soil

efflux agrees closely with the results of a previous girdling experiment in a Swedish boreal forest (Högberg *et al.*, 2001). The Högberg *et al.* (2001) study was carried out in Scots pine forest (*Pinus sylvestris*) with a sparse understorey and approximately double the density of trees compared to the present study. Although there are obvious differences between these ecosystems, our data suggest that mountain birch trees play a disproportionate role in controlling belowground C dynamics in these ecosystems. A girdling treatment in an ericaceous dwarf shrub community (*Calluna vulgaris*) showed no detectable change in soil CO₂ efflux (Kritzler *et al.*, 2016), indicating that roots and associated fungi made a much smaller contribution to total soil

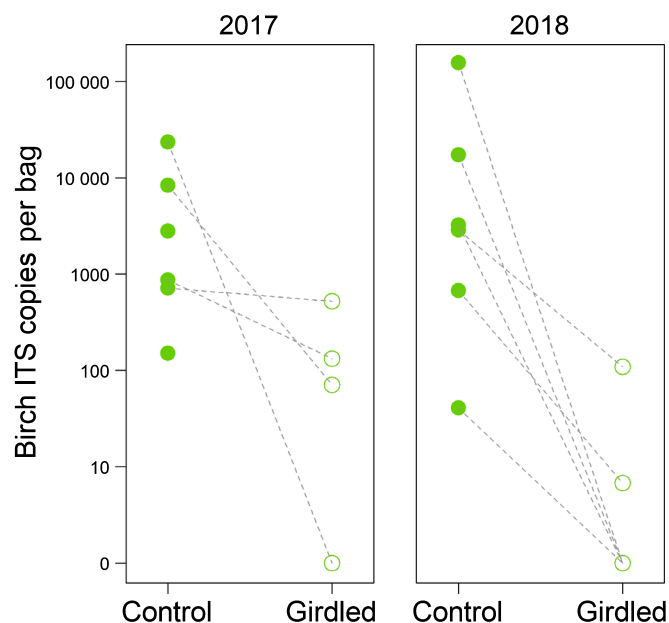


Fig. 4 Birch internal transcribed spacer (ITS) copy numbers in ingrowth bags in paired (denoted by dashed connecting lines) birch girdled and control plots in 2017 and 2018. The results of statistical analyses are shown in Table 2.

respiration than in mountain birch forest. Should trees or shrubs expand onto ericaceous heath, our experiment suggests that the autotrophic component of soil CO_2 efflux would increase disproportionately along with increased GPP.

The reduction in soil efflux of CO_2 in birch plots after girdling coincided with reductions in production of birch roots and mycorrhizal mycelium in birch plots, demonstrating the tight coupling between C assimilation in the canopy, belowground biomass production and return via soil CO_2 efflux. The reduction in birch root and mycelium production was greatest in the second year of the treatment with four of six girdled plots showing zero or near-zero biomass production. This delayed effect suggests that these trees have a degree of resilience to disturbance, potentially in the form of stored nonstructural carbohydrates that can supplement rhizosphere demand in the short term (Palacio

et al., 2008), also supported by some resprouting of shoots below the girdling line. Nevertheless, it is clear that reduction in C supply from the canopy to the rhizosphere resulted in large reductions in soil respiration in both birch and willow plots.

In 2018, despite the large reduction in birch internal transcribed spacer (ITS) copy numbers, there was no significant difference in total root production in the girdled and control plots. We were not able specifically to estimate ericaceous biomass production directly (because of our lack of primers targeting the *Ericaceae*). However, the recovery of overall root productivity, coupled to the major decline in birch ITS copy numbers, strongly suggests that there was an increase in root productivity from the ericaceous understorey plants, most likely as a result of these plants being released from competition with the birch trees. In open birch forests, it is unlikely that shading by the canopy is limiting ericaceous understorey growth; instead, competition for nutrients may exert a stronger control. In support of this explanation, invertebrate herbivore events are known to exert a strong control on canopy productivity in subarctic birch forests (Bjerke *et al.*, 2014) and also increase soil nitrogen availability (Parker *et al.*, 2017), which, along with frass inputs, is suggested to be driven by reduced uptake by the birch canopy (Parker *et al.*, 2017). Such disturbance events may release the understorey from belowground competition and allow for greater ericaceous shrub productivity, as appears to have occurred in our girdling study. Overall, these findings further demonstrate the disproportionate role that birch trees play in driving C and nutrient cycling within these ecosystems, when compared with their contribution to total LAI.

The substantial canopy-linked soil respiration flux integrates a number of processes that occur subsequent to the allocation of photosynthate to the roots. First, roots and their associated mycorrhizal fungi respire as they grow through the soil (Söderström & Read, 1987; Hagenbo *et al.*, 2019). The second potential source of canopy-linked soil CO_2 efflux is positive priming of soil organic matter: greater microbial decomposition of soil C as a result of autotrophic C delivery (Kuzyakov, 2002). Priming previously has been inferred to reduce soil C storage in mountain birch forests compared to tundra heath, despite high aboveground biomass and productivity (Hartley *et al.*, 2012).

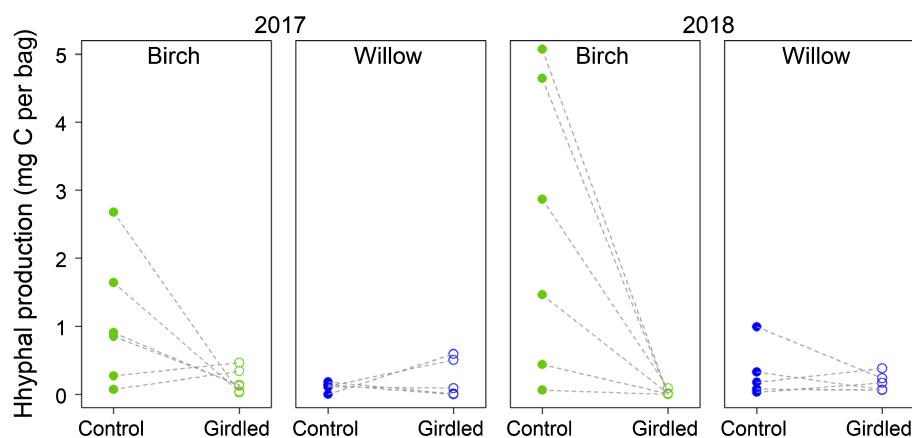


Fig. 5 Hyphal production (mass of carbon (C) into ingrowth bag) in paired (denoted by dashed connecting lines) control and girdled plots in 2017 and 2018 in birch (green) and willow (blue) plots. The results of statistical analyses are shown in Table 2.

Furthermore, ectomycorrhizal (ECM) fungi have been linked to decomposition in boreal, organic-rich soils through the production of extracellular oxidative enzymes (Lindahl & Tunlid, 2015; Sterkenburg *et al.*, 2018; Zak *et al.*, 2019), especially when mineral nitrogen (N) availability is low (Bödeker *et al.*, 2014). Therefore, priming of organic matter by tree and shrub roots and associated mycorrhizal fungi could contribute a significant fraction of the large canopy-linked soil CO₂ efflux.

It is clear that more mycorrhizal hyphae were produced in the birch plots than in the willow plots and that girdling dramatically reduced this production to almost zero. Respiration by mycorrhizal hyphae can contribute from 14 to 26% of total soil CO₂ efflux in boreal forest (Hasselquist *et al.*, 2012; Hagenbo *et al.*, 2019) and is likely to contribute a significant fraction of the canopy-linked flux in our mountain birch plots. Furthermore, nonmelanized mycorrhizal necromass is known to degrade rapidly (Wilkinson *et al.*, 2011; Drigo *et al.*, 2012; Fernandez *et al.*, 2019) and ECM-dominated soils correlate with high soil turnover rates and low soil C compared to ericoid mycorrhizal-dominated systems (Clemmensen *et al.*, 2015; Parker *et al.*, 2015). Therefore, we expect that, in areas of the tundra where soils are dominated by ECM symbioses, fungal symbionts play an important role in the rapid return of autotrophic C as soil CO₂ efflux.

The girdling experiment demonstrates a significant contribution of the willow shrub canopy to soil CO₂ efflux. At its peak, canopy-linked soil CO₂ efflux in willow plots reached 38% of the total flux. Willows belong to a genus of shrubs that are well-documented to be expanding in the Arctic, garnering significant interest in their associated ecosystem feedbacks (Myers-Smith *et al.*, 2011, 2019b). Shrubby ecosystems in the tundra previously have been linked to fast turnover of belowground C (Parker *et al.*, 2015; Sørensen *et al.*, 2018) and leaf litter (Demarco *et al.*, 2014; Parker *et al.*, 2018), but with this experiment we were able to quantify soil CO₂ efflux directly driven by recent canopy C assimilation. We hypothesized that girdling would cause a larger relative reduction in soil CO₂ efflux in birch than in willow plots as a result of higher allocation of C to mycorrhizal networks in the former. Indeed it is clear that more mycelium was produced in birch plots, coinciding with a greater canopy-linked soil CO₂ efflux than from willow plots. However, the fact that this was not statistically significant may be related to the more rapid reduction in LAI within the willow plots.

The limited hyphal colonization of the in-growth bags in both girdled and control willow plots suggests that willow shrubs do not rely significantly on ECM extramatrical mycelium for nutrient acquisition (although colonization by smooth, contact type ECM fungi without extensive mycelial proliferation outside of the roots may take place; Agerer, 2001). As outlined above, this may be due to (a) greater soil moisture in the willow plots, and potential for anoxic conditions, having adverse effects on the fungi (Lodge, 1989; Wurzbürger *et al.*, 2004; Barnes *et al.*, 2018), or (b) drifting snow (Naito & Cairns, 2011) resulting in increased influx of dissolved and particulate compounds and/or increased mobilization of N by the winter-active microbial community (Nadelhoffer *et al.*, 1991; Schimel *et al.*, 2004), and thus

reducing investment in mycorrhizas by the shrubs. We propose that arctic willows, growing typically in moist topographies, may rely more on roots and direct uptake of nutrients, than on ECM fungi.

We have demonstrated that recent photosynthate regulates soil CO₂ efflux in subarctic forest communities beyond what is expected from the contribution of canopies to community LAI. Trees and shrubs are potential future land cover types on what is presently tundra heath (Pearson *et al.*, 2013) and some of the extra C that will be fixed as a result of increasing photosynthesis in these more productive ecosystems will be rapidly returned to the atmosphere through the rhizosphere. Unexpectedly, we found that birch and willow canopies contributed similarly large proportions to soil CO₂ efflux, but much more canopy-fixed C was allocated to mycorrhizal mycelium by birch. At present, our understanding of rhizosphere processes and subsequent C losses lags behind research on aboveground processes. Evidence from previous research suggests that rhizosphere priming of soil organic matter occurs in subarctic treeline forests and that forest expansion could even lead to a net loss of C from the ecosystem (Hartley *et al.*, 2012). The majority of tundra soils have scarce mineral nutrient availability (Shaver *et al.*, 1992), therefore greater investment belowground by plants may be required to mobilize nutrients for further growth. If soil CO₂ efflux increases in tundra soils in response to increased plant growth, a critical research priority will be to understand what proportion of the increased efflux is short-term root respiration, and how much is the decomposition of soil organic matter in response to rhizosphere inputs.

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Author contributions

TCP, PAW, IPH, DJ, BDL, KEC, NLF, LES and J-AS designed, implemented and collected plot-level data from the experiment; JO and MBS collected and processed drone survey data; TCP analyzed data and wrote the manuscript. All authors significantly contributed to multiple drafts of the paper.








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References

- Agerer R. 2001. Exploration types of ectomycorrhizae – a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11: 107–114.
- Barnes CJ, van der Gast CJ, McNamara NP, Rowe R, Bending GD. 2018. Extreme rainfall affects assembly of the root-associated fungal community. *New Phytologist* 220: 1172–1184.
- Bjerke JW, Karlsen SR, Hogda KA, Malnes E, Jepsen JU, Lovibond S, Vikhamar-Schuler D, Tommervik H. 2014. Record-low primary productivity and high plant damage in the Nordic Arctic Region in 2012 caused by multiple weather events and pest outbreaks. *Environmental Research Letters* 9: 084006.
- Bjorkman AD, Myers-Smith IH, Elmendorf SC, Normand S, Rüger N, Beck PSA, Blaggh-Overgaard A, Blok D, Cornelissen JHC, Forbes BC *et al.* 2018. Plant functional trait change across a warming tundra biome. *Nature* 562: 57–62.
- Bödeker ITM, Clemmensen KE, de Boer W, Martin F, Olson Å, Lindahl BD. 2014. Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist* 203: 245–256.
- Bond-Lamberty B, Wang CK, Gower ST. 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? *Global Change Biology* 10: 1756–1766.
- Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* 205: 1525–1526.
- Demarco J, Mack MC, Bret-Harte MS. 2014. Effects of arctic shrub expansion on biophysical vs. biogeochemical drivers of litter decomposition. *Ecology* 95: 1861–1875.
- Drigo B, Anderson IC, Kannangara GSK, Cairney JWG, Johnson D. 2012. Rapid incorporation of carbon from ectomycorrhizal mycelial necromass into soil fungal communities. *Soil Biology and Biochemistry* 49: 4–10.
- Elmendorf SC, Henry GHR, Hollister RD, Bjork RG, Bjorkman AD, Callaghan TV, Collier LS, Cooper EJ, Cornelissen JHC, Day TA *et al.* 2012a. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. *Ecology Letters* 15: 164–175.
- Elmendorf SC, Henry GHR, Hollister RD, Bjork RG, Boulanger-Lapointe N, Cooper EJ, Cornelissen JHC, Day TA, Dorrepaal E, Elumeeva TG *et al.* 2012b. Plot-scale evidence of tundra vegetation change and links to recent summer warming. *Nature Climate Change* 2: 453–457.
- Epstein HE, Reynolds MK, Walker DA, Bhatt US, Tucker CJ, Pinzon JE. 2012. Dynamics of aboveground phytomass of the circumpolar Arctic tundra during the past three decades. *Environmental Research Letters* 7: 015506.
- Fernandez CW, Heckman K, Kolka R, Kennedy PG. 2019. Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming. *Ecology Letters* 22: 498–505.
- Forbes BC, Macias Fauria M, Zetterberg P. 2010. Russian Arctic warming and ‘greening’ are closely tracked by tundra shrub willows. *Global Change Biology* 16: 1542–1554.
- Forrest AF. 2006. *Hybridization in sub-arctic willow scrub in Scotland*. PhD thesis, University of Edinburgh, UK.
- Hagedorn F, Shiyatov SG, Mazepa VS, Devi NM, Grigor'ev AA, Bartysh AA, Fomin VV, Kapralov DS, Terent'ev M, Bugman H, Rigling A *et al.* 2014. Treeline advances along the Urals mountain range – driven by improved winter conditions? *Global Change Biology* 20: 3530–3543.
- Hagenbo A, Hadden D, Clemmensen KE, Grelle A, Manzoni S, Mölder M, Ekblad A, Fransson P. 2019. Carbon use efficiency of mycorrhizal fungal mycelium increases during the growing season but decreases with forest age across a *Pinus sylvestris* chronosequence. *Journal of Ecology* 107: 2808–2822.
- Hagenbo A, Kvaschenko J, Clemmensen KE, Lindahl BD, Fransson P, Wurzbürger N. 2018. Fungal community shifts underpin declining mycelial production and turnover across a *Pinus sylvestris* chronosequence. *Journal of Ecology* 106: 490–501.
- Harsch MA, Hulme PE, McGlone MS, Duncan RP. 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters* 12: 1040–1049.
- Hartley IP, Garnett MH, Sommerkorn M, Hopkins DW, Fletcher BJ, Sloan VL, Phoenix GK, Wookey PA. 2012. A potential loss of carbon associated with greater plant growth in the European Arctic. *Nature Climate Change* 2: 875–879.
- Hasselquist NJ, Metcalfe DB, Högborg P. 2012. Contrasting effects of low and high nitrogen additions on soil CO₂ flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology* 18: 3596–3605.
- Heinemeyer A, Hartley IP, Evans SP, De la Fuente JAC, Ineson P. 2007. Forest soil CO₂ flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology* 13: 1786–1797.
- Heliasz M, Johansson T, Lindroth A, Molder M, Mastepanov M, Friberg T, Callaghan TV, Christensen TR. 2011. Quantification of C uptake in subarctic birch forest after setback by an extreme insect outbreak. *Geophysical Research Letters* 38: L01704.
- Hicks Pries CE, van Logtestijn RSP, Schuur EAG, Natali SM, Cornelissen JHC, Aerts R, Dorrepaal E. 2015. Decadal warming causes a consistent and persistent shift from heterotrophic to autotrophic respiration in contrasting permafrost ecosystems. *Global Change Biology* 21: 4508–4519.
- Hofgaard A, Tommervik H, Rees G, Hanssen F. 2013. Latitudinal forest advance in northernmost Norway since the early 20th century. *Journal of Biogeography* 40: 938–949.
- Högborg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högborg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411: 789–792.
- Iversen CM, Sloan VL, Sullivan PF, Euskirchen ES, McGuire AD, Norby RJ, Walker AP, Warren JM, Wullschlegel SD. 2015. Tansley review the unseen iceberg: plant roots in arctic tundra. *New Phytologist* 205: 34–58.
- Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389–411.
- Kritzer UH, Artz RRE, Johnson D. 2016. Soil CO₂ efflux in a degraded raised bog is regulated by water table depth rather than recent plant assimilate. *Mires and Peat* 17: 1–14.
- Kuhry P, Grosse G, Harden JW, Hugelius G, Koven CD, Ping CL, Schirrmeister L, Tarnocai C. 2013. Characterisation of the permafrost carbon pool. *Permafrost and Periglacial Processes* 24: 146–155.
- Kulmala L, Pumpanen J, Kolari P, Dengel S, Berninger F, Köster K, Matkala L, Vanhatalo A, Vesala T, Bäck J. 2019. Inter- and intra-annual dynamics of photosynthesis differ between forest floor vegetation and tree canopy in a subarctic Scots pine stand. *Agricultural and Forest Meteorology* 271: 1–11.
- Kuzakov Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Für Pflanzenernährung Und Bodenkunde* 165: 382–396.
- Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist* 205: 1443–1447.
- Lodge DJ. 1989. The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in *Populus* and *Salix*. *Plant and Soil* 117: 243–253.
- Myers-Smith IH, Forbes BC, Wilmking M, Hallinger M, Lantz T, Blok D, Tape KD, Macias-Fauria M, Sass-Klaassen U, Levesque E *et al.* 2011. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environmental Research Letters* 6: 045509.

- Myers-Smith IH, Grabowski MM, Thomas HJD, Angers-Blondin S, Daskalova GN, Bjorkman AD, Cunliffe AM, Assmann JJ, Boyle JS, McLeod E *et al.* 2019a. Eighteen years of ecological monitoring reveals multiple lines of evidence for tundra vegetation change. *Ecological Monographs* 89: e01351.
- Myers-Smith IH, Kerby JT, Phoenix GK, Bjerke JW, Epstein HE, Assmann JJ, John C, Andreu-Hayles L, Angers-Blondin S, Beck PSA *et al.* 2020. Complexity revealed in the greening of the Arctic. *Nature Climate Change* 10: 106–117.
- Myers-Smith IH, Thomas HJD, Bjorkman AD. 2019b. Plant traits inform predictions of tundra responses to global change. *New Phytologist* 221: 1742–1748.
- Nadelhoffer KJ, Giblin AE, Shaver GR, Laundre JA. 1991. Effects of temperature and substrate quality on element mineralization in 6 arctic soils. *Ecology* 72: 242–253.
- Naito AT, Cairns DM. 2011. Relationships between Arctic shrub dynamics and topographically derived hydrologic characteristics. *Environmental Research Letters* 6: 045506.
- Palacio S, Hester AJ, Maestro M, Millard P. 2008. Browsed *Betula pubescens* trees are not carbon-limited. *Functional Ecology* 22: 808–815.
- Parker TC, Sadowsky J, Dunleavy H, Subke J-A, Frey SD, Wookey PA. 2017. Slowed biogeochemical cycling in sub-arctic birch forest linked to reduced mycorrhizal growth and community change after a defoliation event. *Ecosystems* 20: 316–330.
- Parker TC, Sanderman J, Holden RD, Blume-Werry G, Sjögersten S, Large D, Castro-Díaz M, Street LE, Subke J-A, Wookey PA. 2018. Exploring drivers of litter decomposition in a greening Arctic: results from a transplant experiment across a treeline. *Ecology* 99: 2284–2294.
- Parker TC, Subke J-A, Wookey PA. 2015. Rapid carbon turnover beneath shrub and tree vegetation is associated with low soil carbon stocks at a subarctic treeline. *Global Change Biology* 21: 2070–81.
- Pearson RG, Phillips SJ, Loranty MM, Beck PSA, Damoulas T, Knight SJ, Goetz SJ. 2013. Shifts in Arctic vegetation and associated feedbacks under climate change. *Nature Climate Change* 3: 673–677.
- Pérez-Izquierdo L, Clemmensen KE, Strengbom J, Nilsson M-C, Lindahl BD. 2019. Quantification of tree fine roots by real-time PCR. *Plant and Soil* 440: 440–593.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2016. [nlme]: Linear and Nonlinear Mixed Effects Models. [WWW document] URL <http://cran.r-project.org/web/packages/nlme/index.html> [accessed 1 April 2019].
- Qian H, Joseph R, Zeng N. 2010. Enhanced terrestrial carbon uptake in the Northern High Latitudes in the 21st century from the Coupled Carbon Cycle Climate Model Intercomparison Project model projections. *Global Change Biology* 16: 641–656.
- R Development Core Team. 2016. *R: A language and environment for statistical computing*. [WWW document] URL <https://www.R-project.org/>.
- Reichle LM, Epstein HE, Bhatt US, Raynolds MK, Walker DA. 2018. Spatial heterogeneity of the temporal dynamics of arctic tundra vegetation. *Geophysical Research Letters* 45: 9206–9215.
- Richardson AD, Friedland AJ. 2009. A review of the theories to explain arctic and alpine treelines around the world. *Journal of Sustainable Forestry* 28: 218–242.
- Rouse J, Haas R, Schell J, Deering D. 1974. *Monitoring vegetation systems in the Great Plains with ERTS*. Washington, DC, USA: NASA.
- Schimel JP, Bilbrough C, Welker JA. 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* 36: 217–227.
- Shaver GR, Billings WD, Chapin FS, Giblin AE, Nadelhoffer KJ, Oechel WC, Rastetter EB. 1992. Global change and the carbon balance of arctic ecosystems. *BioScience* 42: 433–441.
- Shaver GR, Street LE, Rastetter EB, Van Wijk MT, Williams M. 2007. Functional convergence in regulation of net CO₂ flux in heterogeneous tundra landscapes in Alaska and Sweden. *Journal of Ecology* 95: 802–817.
- Siewert MB. 2018. High-resolution digital mapping of soil organic carbon in permafrost terrain using machine learning: a case study in a sub-Arctic peatland environment. *Biogeosciences* 15: 1663–1682.
- Siewert MB, Hanisch J, Weiss N, Kuhry P, Maximov TC, Hugelius G. 2015. Comparing carbon storage of Siberian tundra and taiga permafrost ecosystems at very high spatial resolution. *Journal of Geophysical Research: Biogeosciences* 120: 1973–1994.
- Sjögersten S, Wookey PA. 2002. Climatic and resource quality controls on soil respiration across a forest-tundra ecotone in Swedish Lapland. *Soil Biology and Biochemistry* 34: 1633–1646.
- Söderström B, Read DJ. 1987. Respiratory activity of intact and excised ectomycorrhizal mycelial systems growing in unsterilized soil. *Soil Biology and Biochemistry* 19: 231–236.
- Sørensen MV, Strimbeck R, Nystuen KO, Kapas RE, Enquist BJ, Graae BJ. 2018. Draining the pool? Carbon storage and fluxes in three alpine plant communities. *Ecosystems* 21: 316–330.
- Sterkenburg E, Clemmensen KE, Ekblad A, Finlay RD, Lindahl BD. 2018. Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *ISME Journal* 12: 2187–2197.
- Street LE, Shaver GR, Williams M, Van Wijk MT. 2007. What is the relationship between changes in canopy leaf area and changes in photosynthetic CO₂ flux in arctic ecosystems? *Journal of Ecology* 95: 139–150.
- Street LE, Subke J-A, Baxter R, Dinsmore KJ, Knoblauch C, Wookey PA. 2018. Ecosystem carbon dynamics differ between tundra shrub types in the western Canadian Arctic. *Environmental Research Letters* 13: 84014.
- Sturm M, Schimel J, Michaelson G, Welker JM, Oberbauer SF, Liston GE, Fahnestock J, Romanovsky VE. 2005. Winter biological processes could help convert arctic tundra to shrubland. *BioScience* 55: 17–26.
- Subke J-A, Inglima I, Cotrufo MF. 2006. Trends and methodological impacts in soil CO₂ efflux partitioning: a meta-analytical review. *Global Change Biology* 12: 921–943.
- Sullivan P, Sommerkorn M, Rueth H. 2007. Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. *Oecologia* 153: 643–652.
- Tape K, Sturm M, Racine C. 2006. The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. *Global Change Biology* 12: 686–702.
- Treseder KK. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies. *New Phytologist* 164: 347–355.
- Walker DA, Raynolds MK, Daniëls FJA, Einarsson E, Elvebakk A, Gould WA, Katenin AE, Kholod SS, Markon CJ, Melnikov ES *et al.* 2005. The Circumpolar Arctic vegetation map. *Journal of Vegetation Science* 16: 267–282.
- Wallander H, Ekblad A, Godbold DL, Johnson D, Bahr A, Baldrian P, Björk RG, Kieliszewska-Rokicka B, Kjoller R, Kraigher H *et al.* 2013. Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – a review. *Soil Biology & Biochemistry* 57: 1034–1047.
- Wardle DA, Jonsson M, Bansal S, Bardgett RD, Gundale MJ, Metcalfe DB. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. *Journal of Ecology* 100: 16–30.
- Wilkinson A, Alexander IJ, Johnson D. 2011. Species richness of ectomycorrhizal hyphal necromass increases soil CO₂ efflux under laboratory conditions. *Soil Biology and Biochemistry* 43: 1350–1355.
- Williams M, Bell R, Spadavecchia L, Street LE, Van Wijk MT. 2008. Upscaling leaf area index in an Arctic landscape through multiscale observations. *Global Change Biology* 14: 1517–1530.
- Wilmking M, Harden J, Tape K. 2006. Effect of tree line advance on carbon storage in NW Alaska. *Journal of Geophysical Research-Biogeosciences* 111: G02023.
- Wurzburger N, Hartshorn AS, Hendrick RL. 2004. Ectomycorrhizal fungal community structure across a bog-forest ecotone in southeastern Alaska. *Mycorrhiza* 14: 383–389.
- Yu Q, Epstein H, Engstrom R, Walker D. 2017. Circumpolar arctic tundra biomass and productivity dynamics in response to projected climate change and herbivory. *Global Change Biology* 23: 3895–3907.
- Zak DR, Pelletier PT, Argiroff WA, Castillo B, James TY, Nave LE, Averill C, Beidler K, Bhatnagar J, Blesh J *et al.* 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. *New Phytologist* 223: 33–39.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Example images of plots and study ecosystem.

Fig. S2 Calculated canopy-linked soil CO₂ efflux in birch and willow as a proportion of the total.

Fig. S3 Soil temperature and moisture in birch and willow plots.

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